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Review of the doctoral dissertation presented by Yufeng Guan, entitled „The effect of nitric oxide on histone protein acetylation status in *Phytophthora infestans* (Mont.) de Bary".

The reviewed doctoral dissertation, presented by MSc. Yufeng Guan was completed at the Faculty of Biology, Adam Mickiewicz University in Poznań, under the supervision of Professor Magdalena Arasimowicz Jelonek from the Department of Plant Ecophysiology, Institute of Experimental Biology, Faculty of Biology.

Mr. Yufeng Guan's doctoral dissertation consists of two articles published in recognized international journals: *Plant Physiology and Biochemistry* and *Molecular Plant Pathology*, and one manuscript submitted for publication to *PLOS Pathogens*. The first and third manuscripts described original data obtained from experiments conducted by Yufeng Guan, while the second article provides an overview of the structure, diversity, and phylogeny of histone acetyltransferases (HATs) and histone deacetylases (HDACs) present in various *Phytophthora* species.

The results presented in the articles included in the doctoral dissertation concern biological and molecular studies aimed at explaining the rapid adaptation of *Phytophthora infestans*, one of the most dangerous plant pathogens causing large yields losses, to internal

signals and environmental stressors, including plant-microbe interactions. The presented results explained the high plasticity of *P. infestans* by the epigenetic modification driven by reversible histone acetylation and deacetylation, which play an important role in pathogen gene expression. In addition, Yufeng Guan presented experiments indicating that nitric oxide (NO) and specific reactive nitrogen species (RNS) may function as a regulator of histone acetyltransferase activity that finally enhances adaptability and/or pathogenicity of this harmful plant pathogen .

The presented study is a continuation of the excellent research conducted by the team of Prof. Magdalena Arasimowicz-Jelonek, and previously Prof. Jolanta Floryszak-Wieczorek, on the role and importance of NO and RNS in plant-pathogen interactions. The research presented in the reviewed dissertation is very interesting and innovative from both a scientific and practical perspective. Demonstrating the role of NO and RNS molecules in the epigenetic regulation of pathogen gene expression *via* histone acyltransferases and deacetylases can be considered a scientific novelty. The presented results may guide strategies that could be used in the future to more effectively protect plants against such a dangerous pathogen as *P. infestans*.

The review article entitled “Histone (de)acetylation in epigenetic regulation of *Phytophthora*_pathobiology”, published in *Molecular Plant Pathology* in 2024, provides an excellent introduction to this dissertation topic. It summarizes the available literature on epigenetic regulation of gene expression in oomycetes. Describes the role of histone methylation and discusses in detail the importance of histone acetylation, catalyzed by acyltransferases (HATs), and deacetylation, catalyzed by histone deacetylases (HDACs), in regulating gene expression. The following chapters presented the evolution of HAT and HDAC enzymes in oomycetes, specifically analyzing the exon and intron structure and conserved domains of HAT and HDAC enzymes in *P. infestans*. The final section of the review is devoted to the epigenetic regulation of gene expression in *P. infestans* in response

to the life cycle and changing environmental conditions, especially stresses. In summary, the authors emphasize that the high virulence of *P. infestans* stems from its high adaptability to diverse environmental conditions, which indicated from an epigenetic mechanism based on acetylation/deacetylation of histone proteins, which are capable of modulating gene expression under oxidative stresses and adapting pathogen to the *in planta* conditions.

In the paper published in the journal *Plant Physiology and Biochemistry*, Yufeng Guan's describes the RNS formation and accumulation in *P. infestans* in different developmental stages and during potato-*P. infestans* interaction. Subsequent studies focused on analyzing the patterns of histone H3/H4 acetylation in different developmental stages of virulent and avirulent strains incubated under nitrosative stress conditions. Candidate for a doctorate compared the effect of RNS on the increase of total H3/H4 acetylation and histone acetylation marks in *P. infestans.* The study revealed not only upregulation of acetyltransferase PifHAC3 and PifHAM1, which catalyze H3K56 and H4K16 acetylation, but also an association with the deposition of H3K56 and H4K16 marks on several pathogenicity-related genes, resulting in their increased expression. Finally, the presented study highlights the role of NO and RNS in epigenetic reprogramming of essential gene expression during sporulation and plant-pathogen interaction *via* histone acetylation.

The second original paper, incorporated into this dissertation (presented as a manuscript under revision), Mr. Yufeng Guan demonstrated that the effect of NO and RNS on histone deacetylase (PifHDAC1, PifHDAC3, PifHDAC5) is genotype-specific and can lead to transcriptional reprogramming of *P. infestans,* both avirulent and virulent strains. He identified PifHDAC3 as highly abundant in nitrosative environments and found that genotype-specific redistribution of PifHDAC3 binding demonstrates epigenetic flexibility associated with the pathogen's virulence pattern. Finally, using chromatin

immunoprecipitation sequencing, he was able to demonstrate that NO-dependent redox changes modulate PifHDAC3 binding at the Avr3a effector locus, contributing to the increase of virulence. The results presented in this manuscript indicated that interplay between RNS and histone deacetylases is crucial in influencing the expression of pathogenicity-related genes (*CesA1, sPLD-like1, PiCAT2*). This suggests that NO/RNS may disrupt pathogen virulence through its effect on PifHDAC3 activity.

Referring to the conclusions formulated in this publication, concerning the possibility of using the described mechanism of the effect of nitrosative stress on epigenetic regulation of gene expression, to develop a strategy for breeding plants resistant to *P. infestans*, I would like to ask the Candidate genetic what kind of modification/s you could recommended to breeders who would like to obtain potato cultivars resistant to this pathogen?

The submitted manuscript has been carefully prepared and is expected to be published soon. The lack of titles for the figures presented in the publication poses a certain difficulty; only the titles of the figures included in the supplement are included. However, this is merely a technical deficiency that does not detract from the publication's value.

On the basis of the presented studies the Mr. Yufeng Guan concluded that nitric oxide and nitrogen species are engaged not only in diverse regulatory processes, but also function as an epigenetic modulator through histone acetylation and regulation of gene expression, and affect developmental processes and responses to stressful environmental conditions. It is worth to underline, that the dissertation contained very well design diagrams illustrating: 1) the influence of NO on epigenetic control of critical pathogenicity-related gene expression *via* histone H3/H4 acetylation during virulent *P. infestans* life cycle, 2) overview of currently known epigenetic modifications observed in *Phytophthora* species and the proposed potential impact of nitro-oxidative stress on their lifestyle and

pathogenicity, 3) influence of bioavailability of NO during potato - Avr/vr *P. infestans* interaction on PifHDAC3 displacement from the *Avr3a* promoter.

The reviewed dissertation indicated that the candidate for a doctorate presents theoretical and practical knowledge in the molecular biology, especially in the context of epigenetic regulation of gene expression during the plant - pathogen interactions and the ability to independently conduct scientific work. The reviewed doctoral dissertation demonstrated an original solution of a scientific problem and a possible application of the knowledge about the influence of nitrosative stress on acetylation of histones and epigenetic regulation of the expression of *P. infestans* virulence genes on efficient control of plants against this harmful pathogen.

In conclusion, I assess that the research objectives of Yufeng Guan's doctoral thesis were achieved and the obtained results are scientifically valuable, detailed described and properly discussed. Mr. Yufeng Guan showed the ability to apply different microbiological, biochemical, molecular and bioinformatics methods and indicated conscientiousness and perseverance when carrying out multiple research tasks.

I express my appreciation to Msc. Yufeng Guan and his supervisor, Prof. Magdalena Arasimowicz-Jelonek for a very interesting and valuable doctoral dissertation. The he presented dissertation perfectly documents the scientific skills and experience of Yufeng Guan, which will enable him to conduct important biological and molecular studies in an international scientific environment in the future. I would like to underline that the PhD candidate already published a big part of his dissertation, and the last part is ready for publication (is currently under revision).

Conclusions

In my opinion , the PhD thesis of Yufeng Guan presented for review meets all the requirements set out in the art. 187 of the Act of 20 July 2018, The Law on Higher

Education and Science. I hereby recommend to the Scientific Council of Biological Sciences Discipline of the Faculty of Biology, Adam Mickiewicz University of Poznań, to admit Mr. Yufeng Guan to public defense of his thesis and further stages of the doctoral procedure.

At the same time, in recognition of a wide range of research and valuable published results, I recommend awarding the thesis with an appropriate prize.

Wnioski końcowe

W świetle przedstawionej powyżej, bardzo pozytywnej oceny rozprawy doktorskiej Pana magistra Yufeng Guan, wnioskuję do Rady Naukowej Dyscypliny Nauki Biologiczne Wydziału Biologii Uniwersytetu Adama Mickiewicza w Poznaniu o dopuszczenie Go do dalszych etapów postępowania doktorskiego.

Recenzowana praca została spełnia warunki określone w art. 187 Ustawy Prawo o szkolnictwie wyższym i nauce z dnia 20 lipca 2018 r. (Dz. U. z 2024 r. , poz. 1571) o stopniach naukowych i tytule naukowym

Z pełnym przekonaniem stawiam wniosek o wyróżnienie bardzo wartościowej rozprawy doktorskiej Pana mgr. **Yufeng Guan.** *Mój wniosek uzasadniam wysoką wartością naukową recenzowanej rozprawy oraz opublikowaniem wyników badań przedstawionych w rozprawie w międzynarodowych czasopismach .*